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Kunzmann, A. T., Graham, S., McShane, C. M., Doyle, J., Tomassino, M., Johnston, B., Jamison, J., James, J. A., McManus, D., & Anderson, L. A. (2017). The prevalence of viral agents in esophageal adenocarcinoma and Barrett's esophagus: A systematic review. *European Journal of Gastroenterology and Hepatology*.
<https://doi.org/10.1097/MEG.0000000000000868>

Published in:
European Journal of Gastroenterology and Hepatology

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
[Link to publication record in Queen's University Belfast Research Portal](#)

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Not final version

The prevalence of viral agents in esophageal adenocarcinoma and Barrett's esophagus: A systematic review

Short title: Viral agents & esophageal cancer review

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Source of funding: Cancer Research UK Grant Award (C21244/A19029).

Conflicts of interest: None declared.

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AT Kunzmann, S Graham, CM McShane, J Doyle, M Tommasino, B Johnston, J Jamison, JA James, D McManus & LA Anderson (In Press) The prevalence of viral agents in esophageal adenocarcinoma and Barrett's esophagus: A systematic review. European Journal of Gastroenterology & Hepatology.

ABSTRACT

Background & Aims: Human papillomavirus (HPV), which may reach the esophagus via orogenital transmission, has been postulated to be associated with esophageal adenocarcinoma. A systematic review of the literature investigating the prevalence of infectious agents in esophageal adenocarcinoma and Barrett's esophagus was undertaken.

Methods: Using terms for viruses and esophageal adenocarcinoma, the Medline, Embase and Web of Science databases were systematically searched for studies published, in any language, until June 2016 that assessed the prevalence of viral agents in esophageal adenocarcinoma or Barrett's esophagus. Random effects meta-analyses of proportions were used to calculate the pooled prevalence and 95% confidence intervals (CI) of infections in esophageal adenocarcinoma and Barrett's esophagus.

Results: A total of 30 studies were included. The pooled prevalence of HPV in esophageal adenocarcinoma tumour samples was 13% (n=19 studies, 95% CI: 2-29%) and 26% (n=6 studies, 95% CI: 3-59%) in Barrett's esophagus samples. HPV prevalence was higher in esophageal adenocarcinoma tissue than in esophageal tissue from healthy controls (n=5 studies, pooled odds ratio=3.31, 95% CI: 1.15-9.50). The prevalence of Epstein-Barr virus (EBV) in esophageal adenocarcinoma was 6% (n=5, 95% CI: 0-27%). Few studies have assessed other infectious agents. For each of the analyses, considerable between-study variation was observed ($I^2=84-96\%$), however sensitivity analyses did not reveal any major sources of heterogeneity.

Conclusions: The prevalence of HPV and EBV in esophageal adenocarcinoma is low compared to other viral associated cancers but may have been hampered by small sample sizes and detection

methods susceptible to fixation processes. Additional research with adequate sample size and high quality detection methods is required.

Keywords: Infectious agents; esophageal adenocarcinoma; Barrett's esophagus; systematic review

BACKGROUND/INTRODUCTION

In recent years the incidence of esophageal adenocarcinoma (EAC) has risen by up to 81% within Western societies [1,2]. While the aetiology is not fully understood, obesity, smoking and gastroesophageal reflux have been linked previously to the development of EAC [3–5].

EAC results from a sequence of pathological transformations postulated to begin with reflux esophagitis progressing to Barrett's esophagus (BE) and later to dysplasia. Triggers for progression through this pathway have not yet been identified [6]. BE is an established premalignant lesion of the esophagus, where squamous epithelium is replaced by columnar epithelium [7]. Associated with a 0.5% annual risk of progression to EAC, BE patients undergo regular surveillance via endoscopy and biopsy [8,9]. Survival rates for EAC patients remains low [10] and therefore identifying risk factors for the progression of BE could lead to improved patient outcomes by implementing possible preventative strategies [11].

Suppression of T and B cells has been reported in BE patients and abnormally rapid progressions from BE to EAC can occur following immunosuppressive therapy [12–15]. This would suggest that an impaired immune system and/or infectious agents may play a role in the development of EAC. Infectious agents including hepatitis, Epstein Barr Virus (EBV), Human Herpes Virus 8 (HHV8), merkel cell polyoma virus *Helicobacter Pylori* (*H.Pylori*) and Human Papillomavirus (HPV) cause up to 20% of cancers worldwide [16]. Although the mechanisms are not fully understood, viral agents are believed to contribute to cancer development by inducing chronic inflammation, DNA damage, telomere shortening and cellular proliferation [17]. Chronic inflammation has an established role in BE/EAC development [18].

Most research surrounding the relationship between viral infectious agents and BE/EAC has centred on HPV infections [19,20]. HPV, which may reach the esophagus via orogenital transmission [21] has previously been linked with a variety of cancers including cervical squamous cell cancer, a range of head and neck cancers and esophageal squamous cell carcinoma [20,22,23]. Although several studies have investigated HPVs relationship with EAC, the association remains inconclusive. A systematic review on the association between HPV and EAC was conducted and found a prevalence of 35%, though this estimate may change as key studies have been published since [20]. One study in Australia by Rajendra et al reported HPV prevalence in EAC to be 66.7% which was almost 3 times higher than hospital controls, however these findings have not yet been replicated [24].

Other agents such as JC virus, EBV, Cytomegalovirus (CMV) and adenovirus 12 have been investigated to some degree but findings have been limited by sample size [25–28].

Although HSV1 has been shown to increase the occurrence of gastroesophageal reflux [29], raising the possibility of a role in progression, though to our knowledge no studies have investigated it's relationship to EAC.

The aim of the current study was to systematically review all studies that assessed the prevalence of infectious agents in EAC and BE and to estimate pooled prevalence for the infectious agents using meta-analyses.

METHODS

LITERATURE SEARCH

To investigate the prevalence of viral agents in EAC and BE, three databases including MEDLINE (1947-), EMBASE (1974-) and PUBMED (1946-) were systematically searched from inception to 12th June 2016 to identify primary studies, published in any language. Databases were searched using exploded subject headings and combinations of keywords for EAC/BE such as “(o)esophageal adenocarcinoma”, “(o)esophageal cancer”, “(o)esophageal carcinoma”, “(o)esophageal neoplasm”, “Barrett’s (o)esophagus”, “Barrett’s metaplasia”, “intestinal metaplasia” and combined with exploded subject

headings and keywords for viruses such as “virus”, “viral”, and specific oncogenic virus names. Cross referencing was used to find additional studies that may be relevant. A full list of search terms is presented in Appendix 1.

ELIGIBILITY CRITERIA

Titles and abstracts of articles identified through the search were reviewed independently, in duplicate, by a primary reviewer (SG) and one of three reviewers (CM, AK, JD). Articles identified by any investigator for possible inclusion were then independently reviewed by two other investigators. If there was disagreement between the reviewers, an independent review was undertaken by a third reviewer and a consensus reached. Authors of studies deemed to be of interest but for which insufficient data was reported in the paper were contacted by email.

The inclusion criteria for the meta-analysis were observational studies in which viral DNA or RNA were sought within the affected tissues of the esophagus at diagnosis or prior to the development of EAC. **We also included studies in which viral antibodies were sought within serum/plasma in patients with BE or EAC but only when a comparison to healthy individuals was available, as this could inform differences in exposure.** Association studies addressing the risk between viral infection and risk of EAC were also considered.

Case reports, review articles, letters and non-human studies were excluded from the search. Articles looking only at esophageal squamous cell carcinomas or esophago-gastric junction tumours or where results for esophageal adenocarcinomas were not provided separately were excluded. Articles only featuring viral infection onset after EAC/BE diagnosis or reporting the prevalence of cancer amongst infected patients rather than of infections within cancer patients were also excluded.

If multiple eligible versions of a study were found, data from the article with the largest relevant sample size were included.

No language restrictions were imposed, and articles in non-English languages were translated where necessary.

DATA EXTRACTION

A proforma was developed and the data extracted from the relevant studies by AK and checked for validity by LA. Information extracted from the studies included first author surname, year of study, study location, recruitment period, age range and median/mean, gender ratio, methodology used to identify patients, methodology of infection assessment including primers used for polymerase chain reaction (PCR), type(s) of infection examined, the number of infection positive and negative individuals with adenocarcinoma, BE or comparison healthy tissue/individuals and the types of HPV identified (Table 1).

Study quality was judged using a 7-item scale based on the Joanna Briggs Institute scale for use in systematic reviews addressing questions of prevalence [30] and the STROME-ID for epidemiological infection studies [31] by AK and MT. The items used related to the representativeness of the sample to the target population, the appropriateness of participant recruitment, the adequacy of the sample size, coverage of data analysis sample, reliability of the method (Genotyping/hybridisation of PCR products), use of appropriate controls in PCR, and use of methods to minimise cross-contamination.

STATISTICAL ANALYSIS

Stata 14 software (Stata Corporation, College Station, TX, USA) was used for data analysis. Random effects meta-analysis of proportions, with the Freeman-Tukey double arcsine transformation to avoid issues with zero values, was used to examine the association between each infectious agent and BE and EAC, separately, when 4, or more, studies were eligible. Random effects meta-analyses were used to examine the pooled odds ratio for infections in individuals with and without EAC. A chi-squared test for heterogeneity was calculated and the I^2 statistic determined, to estimate the proportion of variation between study results attributable to heterogeneity rather than chance [32]. Heterogeneity was considered high if I^2 statistic was above 75% [32]. Sensitivity analyses were conducted to examine

the prevalence by study size (<20 versus ≥ 20), study quality (<5 versus ≥ 5), type of tissue (Frozen versus formalin-fixed paraffin embedded samples), **timing (>2006 v ≤ 2006)** and geographical location of study. Sensitivity analyses to examine causes of heterogeneity were examined by removing outliers and each study individually in turn. Due to a limited number of studies, publication bias could not be assessed [33].

RESULTS

STUDIES INCLUDED

The literature search yielded a total of 8099 citations (Embase n=4802; Medline n=1782; PubMed n=1515, figure 1). After removal of duplicates, 6191 potentially relevant citations remained. After application of the exclusion criteria to the titles and abstracts, 170 relevant studies were identified of which 30 met the inclusion criteria[24–28,34–54]. For study characteristics see table 1.

HPV PREVALENCE IN INDIVIDUALS WITH EAC

In total 19 studies [24,27,34–36,38,40–42,45,47–49,51,53–57], including 536 (mean n=28.2, range 1-201) individuals with EAC, examined the prevalence of HPV in EAC tissue (n=19). The pooled prevalence of HPV in tissue from individuals with EAC was 12.5% (95% CI: 1.5-28.8%, Figure 2). A high degree of heterogeneity was observed ($I^2=92.6\%$). Removal of individual studies did not alter heterogeneity ($I^2=88.2-92.4\%$). The prevalence of HPV was similar after exclusion of studies with a sample size of less than 20 EAC patients (n=11, pooled prevalence=13%, 95% CI: 1-33%) and heterogeneity remained high ($I^2=95.3\%$).

PCR was the most widely used method to assess HPV prevalence in tissue. HPV prevalence was similar between studies with higher quality assessment scores (≥ 5) (n=7, pooled prevalence=11%, CI: 0-38%) and studies with lower quality assessment scores (<5) (n=12, pooled prevalence=14%, 1-34%) and

heterogeneity remained high (95.8% and 84.4%, respectively). HPV prevalence was similar between studies using frozen tissue samples (n=6 studies, pooled prevalence=18%, 95% CI: 0-54%) and formalin fixed tissue samples (n=10, pooled prevalence=10%, 95% CI: 0-28%) for DNA analysis, with high heterogeneity within both ($I^2=92.0$ and 91.5% , respectively). **HPV prevalence did not differ by time, with prevalence in studies in the last decade (after 2006) (n=10 studies, pooled prevalence=13%, 95% CI: 1-33%) similar to prevalence in studies prior to this (n=9 studies, pooled prevalence=13%, 95% CI: 0-44%).** For comparison, HPV prevalence was similar in 2 further studies examining HPV prevalence in serum from EAC patients [46,52] (n=2, pooled prevalence=18%, 95% CI: 10-28%).

No statistically significant geographical differences were noted: though the pooled prevalence of HPV in EAC patients appeared modestly higher in North American studies (n=7, pooled prevalence=19%, 95% CI: 0-51%) than elsewhere (n=12, pooled prevalence=9%, 95% CI: 0-26%).

Too few studies adequately reported results by HPV genotypes to assess HPV genotype specific prevalence.

Five studies that assessed the prevalence of HPV in EAC serum or tissue also assessed prevalence in individuals without esophageal conditions. HPV prevalence was higher in individuals with EAC than in individuals without esophageal conditions (pooled OR=3.31, 95% CI: 1.15-9.50, Figure 3), though heterogeneity was present ($I^2=58.9\%$).

There was no difference in HPV prevalence in serum samples between individuals with EAC and individuals without esophageal conditions (n=2, pooled OR=1.55, 95% CI: 0.72-3.35, $I^2=0\%$). Exclusion of studies using serum samples increased the odds ratio (n=3, pooled OR=7.61, 95% CI: 3.30-17.54) and lowered heterogeneity ($I^2=0\%$).

HPV PREVALENCE IN INDIVIDUALS WITH BARRETT'S ESOPHAGUS

Six studies [24,34,39,40,43,58] examined the prevalence of HPV in tissue from a total of 340 (mean=56.7, range=4-112) individuals with BE. The pooled prevalence of HPV in individuals with BE

was 26% (95% CI: 3-59%). A high degree of heterogeneity was observed ($I^2=96.8\%$). Removal of individual studies did not alter heterogeneity ($I^2=94.4-97.5\%$). The heterogeneity remained high after stratification by use of frozen or fixed tissue samples and amongst studies with moderate quality assessment scores (>4 , only one study had a high quality assessment score ≥ 5). We were unable to investigate the effect of primer type or geography due to a limited number of studies. Too few studies adequately reported results by HPV genotypes to assess HPV genotype specific prevalence.

Too few studies were available to compare HPV prevalence in individuals with BE to prevalence in individuals with EAC or without esophageal conditions.

EBV PREVALENCE IN INDIVIDUALS WITH EAC

Five studies [27,28,37,44,50] examined the prevalence of EBV in tissue or serum from a total of 310 (mean=62, range=13-112) individuals with EAC. The pooled prevalence of EBV in individuals with EAC was 6% (95% CI: 0-27%, figure 5). A high degree of heterogeneity was observed ($I^2=91.8\%$). The estimate and degree of heterogeneity were similar after exclusion of studies ($n=1$) with less than 10 EAC cases (pooled prevalence=9%, 95% CI: 0-31%, $I^2=93.8\%$).

The prevalence was higher in studies using PCR to assess EBV prevalence ($n=2$ studies, pooled prevalence=25%, 95% CI: 14-38%), whereas no cases of EBV were detected in three studies solely using in-situ hybridization.

PREVALENCE OF OTHER INFECTIOUS AGENTS

Only two studies examined the prevalence of adenovirus infection [26,27], with neither study finding any positive samples in 2 or 17 EAC patients, respectively. Only 1 study examined JCV [25], and found 22% (2 from 9) of EAC samples and 0% (0 from 20) BE samples were positive. Only 1 study examined cytomegalovirus [27] with none of the 17 EAC samples positive. No studies examining other infectious agents were identified.

DISCUSSION

In the most comprehensive systematic review of the prevalence of infectious agents in EAC and BE samples to date, the prevalence of HPV infection was estimated to be around 12% in individuals with EAC, around 26% in individuals with BE and only 7% (0-18%) in healthy esophageal tissue. The prevalence of EBV in individuals with EAC was low at around 6% (0-27%). However, there was considerable heterogeneity in study estimates and study quality was generally low.

The estimated prevalence of HPV in EAC (12%) is slightly lower than the estimate from a previous systematic review containing only 5 studies (35%) and the estimated prevalence within esophageal squamous cell carcinoma (22.2%). The prevalence is also considerably lower than previous estimates of the prevalence of HPV infection in squamous cell carcinoma of the uterine cervix and adenocarcinoma of the uterine cervix (~83.5%) [59], indicating that should any causal role in EAC exist, it is likely to be less dominant than the role of HPV in cervical cancer [60]. A less dominant role is perhaps to be expected as the orogenital transmission route may be particularly limited for lower parts of the esophagus, where EAC is common.

A comparison within studies assessing HPV prevalence in samples from individuals with EAC and individuals free from cancer using the same methodology suggested the prevalence of HPV was significantly higher in EAC samples. This adds to research not included in the review which found that HPV viral load was increased along the dysplasia-metaplasia-adenocarcinoma pathway [61], and found that persistence of HPV after ablation for BE was associated with dysplasia [60,62]. In addition, evidence from HPV transgenic mouse models indicates these mice are more susceptible to esophageal cancer [63]. Therefore, the role of HPV infections in EAC warrants further study as a possible causative factor. However, these studies were cross-sectional and adjusted estimates were not available so a temporal association free from confounding cannot be confirmed. Studies within individuals examining HPV presence or viral load throughout the dysplasia-metaplasia-adenocarcinoma may help examine a temporal association.

The slightly higher prevalence of HPV in BE was not statistically different from the prevalence in EAC and too few studies assessed both EAC and BE to assess within-study differences in HPV prevalence. It is possible that the slightly higher estimate for the prevalence of HPV infection in BE could be an artefact of different study methods/populations. Whilst the lack of a higher prevalence of HPV infections in EAC compared to BE could indicate a lack of a causal role in progression, it is possible that HPV contributes to malignant changes early in the development of BE which may continue even after immune clearance. A comparison of the prevalence of infectious agents, such as HPV, in progressors from BE to EAC versus non-progressors would help examine a role of infectious agents in the development of EAC.

The prevalence of EBV in the current study appears lower than that of HPV and could indicate a lack of a role of EBV in EAC. However, studies have been small to date and one study found prevalence was as high as 47% [27] indicating that further studies using larger sample sizes and thorough anti-contamination procedures are warranted.

The current systematic review appears to be the first to examine the prevalence of infections in BE. To our knowledge it is also the most comprehensive review of the prevalence of infections in EAC, including 30 studies of which 19 studies (536 individuals) were included in the meta-analysis for HPV in EAC versus only 5 in a previous meta-analysis [20], including additional studies with high quality assessment scores (>5).

The review however highlights a number of limitations of the current literature. The individual studies in the literature to date have mostly had limited sample sizes with a mean sample size of less than 30. All but one of these studies, with a sample size of 201, may have provided too small a sample to adequately estimate the prevalence in the target population, as a power calculation, $n = (1.96^2 \times \text{expected prevalence}(1 - \text{expected prevalence})) / 0.05^2$ [30] where the expected prevalence = 0.13, indicates that a sample size of 174 would be needed to adequately assess prevalence in the target population. Therefore the estimate and the level of heterogeneity may change if

adequate sample sizes were available. **We decided to include the smaller studies, despite the limitations acknowledged above, to provide a more comprehensive assessment of the state of the existing literature.**

The heterogeneity of study results was also considerable ($I^2=92.6$). Attempts to identify the source of heterogeneity via sensitivity analyses, including by geography, study quality, study size and removing individual studies/outliers, were unsuccessful, suggesting the limited sample size of the studies may be responsible. The high heterogeneity could also be due to the variation in HPV detection methods used, as HPV detection may be sensitive to storage times, primer lengths and small procedural changes where contamination is possible [64]. The majority of studies used formalin fixed tissue, often from samples collected over 10 years prior to analysis, for HPV DNA detection, where DNA degradation may occur which could result in lower sensitivity of PCR amplification of long DNA fragments. Whilst sensitivity analysis did not indicate considerable differences between studies using formalin fixed tissue or frozen tissue, it is still possible that small procedural differences or storage times contributed to the high heterogeneity and may have hampered estimation of HPV prevalence.

Too few studies reported results for each HPV type to assess prevalence of HPV types in EAC or BE patients. Similarly, too few studies examined the role of other infectious agents in EAC or precursor lesions. It is also possible that multiple infections may give rise to EAC, meaning an analysis of only one infection type is insufficient in examining the proportion of EAC tissue infected by any type of infectious agent.

In summary, whilst the literature to date indicates that HPV prevalence in EAC samples was higher than in samples from healthy controls, the prevalence is low. The estimates were highly variable, which could be a result of limited sample sizes and variations in detection methods. Future studies using larger sample sizes, better detection methods and examining a broad range of infectious agents and HPV types are needed to determine the true prevalence of infectious agents in EAC.

Table and Figure legends

Table 1: Study characteristics

Table 1: continued...

Figure 1: Study selection

Figure 2. Forest plot of HPV prevalence in esophageal adenocarcinoma tissue.

Figure 3. Forest plot comparing HPV prevalence in individuals with esophageal adenocarcinoma compared to individuals without esophageal conditions.

Figure 4. Forest plot of HPV prevalence in Barrett's esophagus tissue

Figure 5. Forest plot of EBV prevalence in esophageal adenocarcinoma tissue.

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